IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

LINDQVIST et al — Atty. Ref.: 117-357

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DECLARATION

The Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir

1. I, Kevin FitzGerald, do hereby declare and state as follows:

2. I am a UK citizen of Isogenica Limited of Babraham Hall, Babraham, Cambridge CB2 4AT, United Kingdom. I have the qualifications of Ph.D (University of East London). My current position is (Chief Executive Officer) and I have held this position for 3 years. A copy of my curriculum vitae is attached.

3. I am an employee of Isogenica Limited, the assignee in the above-identified application. I have read and am familiar with the above-identified application, the Office

Action mailed on 4 November 2002 and the references cited therein.

4. I have been asked to comment on the content of the description provided in the above-identified application and, in particular, whether that description provided me with sufficient information to reproduce the claimed invention using a *cis*-acting protein other than the protein P2A. I can confirm that given the description and the general knowledge available in February 1997, the substitution of alternative covalent *cis*-acting DNA binding proteins for P2A is a matter of routine.

5. I have been involved with the use polypeptide screening technologies, in order to identify ligands to a given target, for more than 11 years. The first technique that was developed for screening polypeptide libraries involved the ligation of gene libraries into a phage vector (typically lambda) followed by transformation or transfection of *E.coli* and then spatially arraying individual colonies on an agar culture plate. In order to identify the gene sequence that encoded a polypeptide of interest (e.g., a ligand to a target compound), each individual colony (which harboured a unique gene) had to be assayed for the presence of the required expressed polypeptide. With this method, addressing more than between 100,000 and 1,000,000 clones was impractical i) due to the physical constraints of arraying a larger number of discrete colonies on agar plates and ii) due to the limitations of the screening methods themselves (the limited throughput of filter lifts, plaque assays etc.).

6. It has been well understood for many years that increasing the size and diversity of a gene expression library would result in the isolation of higher affinity/finer specificity ligands. Due to the impracticalities of scaling up the direct screening approaches as discussed above, a new concept was conceived in the mid 1980s in which the screening of individual colonies in order to identify those expressing polypeptides of interest was replaced by an affinity selection method. The main advantage of this was that the constraints on addressable library sizes that restricted the lambda library approach were immediately overcome. This new concept involved the physical linkage of each gene within a library with its encoded polypeptide. This meant that by incubating the entire library pool with an immobilised target, it was possible to affinity purify those rare library members that bound to the target and the co-purification of the encoding gene and the encoded binding ligand enabled recovery and isolation of the encoding gene. By replacing the screening method with an affinity selection method, addressing libraries larger than 1,000,000 genes became facile.

7. The technique that exemplified this concept was phage display. I have extensively used the phage display technology and I, therefore, have full understanding of the significance of linking a gene sequence with its encoded polypeptide in order to apply affinity selection techniques to the identification of ligands from diverse libraries. I further understand that the substitution of different phage species for the prototype phages used in the first exemplification of phage display (M13 or Fd), would be

eminently possible. Indeed, there have been many reports in the literature of the implementation of lambda phage, T7 phage and P2 phage for the display and selection of polypeptide ligands. This successful implementation of alternative phages is due to the fact that it is the functional linkage of a gene (encased within the phage virion) and the expressed polypeptide (fused to a phage coat protein) that is the fundamental requirement of the technology and not the individual characteristics of the phage itself. Indeed, other non-phage biological particles have been used to achieve this linkage: bacterial cell surface display, eukaryotic viral display (e.g., retroviral display) and yeast surface display have all been reported in the literature.

8. One major disadvantage of these display technologies is that the production of the gene-polypeptide linkages required the insertion of the gene library into a living cell - for phage display *E.coli* cells are used. This cellular transformation step imposes a size limit of about 10^{10} to the number of genes that these libraries can contain (simply due to the efficiency with which cells can be transformed with recombinant DNA - the 'transformation efficiency'). In order to increase further the size of display libraries, the linkage between gene and polypeptide therefore needs to be achieved *in vitro* (i.e., without the need to transform living cells).

9. The present invention, referred to as covalent display exploits a class of proteins that are able to covalently bind, spontaneously, to their own encoding

DNA (i.e., *in cis*) during the processes of transcription and translation. The basic idea is described on page 4-5 of the above-identified patent application: "*Covalent DNA:protein expression libraries of the invention are made possible by the inclusion of a sequence within the genetic material which encodes a protein which binds covalently to its own encoding DNA and which includes, or is overlapping or adjacent to, the coding sequence for the peptide or protein for display. When expressed, the DNA-binding protein and the display peptide or protein form as a single polypeptide, which becomes covalently attached to the encoding DNA*". Assuming that the interaction between each gene and its expressed polypeptide occurs *in cis*, the production of such complexes can occur within the single compartment of an *in vitro* transcription/translation medium, no prior compartmentalisation through transformation of a living cell culture is required. Hence, any protein that is able to bind covalently to its own DNA *in cis* and to which a library of peptides for display can be fused, can facilitate the physical linkage between gene and polypeptide that is necessary for affinity selections to be performed.

10. The prototype protein for the present invention is the replication protein from P2 phage which is called P2A (it is the product of the A gene of phage P2). As with the implementation of different phage species to enable phage display selections to proceed (as described above), it is clear that any protein that has functional equivalence to P2A (i.e., in its ability to bind covalently *in cis* to its own encoding DNA) would be equally able to link a gene to its encoded polypeptide *in vitro* and facilitate the

generation of polypeptide libraries addressable through affinity selection. All that is required is that a pool of DNA templates is produced, where each template contains a promoter followed by a gene encoding the *cis*-acting protein ligated in frame to a sequence encoding the peptide for display. These constructs are then transcribed and translated *in vitro* to produce the pool of DNA:protein complexes ready for affinity selection.

11. On page 10 of the patent application, a number of alternative proteins (the replication initiation proteins from phage 186, HP1, PSP3 and phiX174) that exhibit similar properties to P2A are disclosed. It is clear that any of these, or indeed any other functionally equivalent protein, can be used to replace P2A as the linking protein of the invention. To demonstrate this, we have made peptide libraries in which phiX174 A-protein as well as a truncated variant of this protein, phiX174A*, have been used in place of P2A to construct polypeptide libraries and we have successfully used such libraries for the selection of peptide ligands.

12. It is well known that these proteins bind covalently to replicative form viral DNA (Dubeau L, Denhardt DT. 1981 The mechanism of replication of X 174. XVIII. Gene A and A* proteins of X 174 bind tightly to X 174 replicative form DNA. Biochim Biophys Acta 653: 52-60). In this example, we appended tac promoter sequence to the 5'-end of the X174a gene and a library of random 12mer peptide sequences to the 3'-end of the gene. The DNA was transcribed and translated *in vitro* in *E.coli* S30 extracts. The library was selected against an anti-ACTH peptide hormone antibody, to recover

any peptides and encoding DNA from the anti-ACTH peptide hormone antibody. DNA is then recovered by PCR, and the selection repeated. The DNA encoding selected peptides is then cloned out and antibody binding sequences identified by ELISA (see figure 1).

13. Selection with X174A libraries is carried out by the following steps:

1) Transcription/translation reaction. 1x200µl ITT reaction was set up per library using Promega lysate for linear templates (Lot 143346) 5. 10µl DNA (2.5µg), 20µl 2.5x buffer, 0.5µl 100mM methionine, and 15 µl S30 extract (thawed rapidly then placed on ice), are mixed and incubated at 25C for 1 hour and used immediately for selection. In round 2 and subsequent rounds of selection, the amount of DNA in the ITT reaction is reduced to 5µg.

2) Solid phase selection. NUNC Star immunotubes are coated overnight with anti-human ACTH antibody at 30µg/ml PBS (2ml/tube, amount reduced to 25µg/ml for subsequent rounds) at 4C, then rinsed 2x PBS (fill & empty). Tubes are blocked at room temperature for 1 hour on the blood mixer with Superblock/0.2mg/ml herring sperm DNA, then rinsed 2xPBS. 90% of library ITT diluted 1:10 in 2ml superblock/0.1% Tween 20/ 0.2mg/ml herring sperm DNA and incubated for 2 hours at 4°C (reduce to 1 hour for round 2). 5% was used for positive control selection in 2ml incubation buffer in a leptin coated immunotube, and 5% for negative control selection in an uncoated immunotube. Tubes are washed 3x PBS/0.1% Tween 20, then 1x superblock/0.1% Tween 20/0.2mg/ml herring sperm DNA for 30

minutes at 4°C, then 3x PBS/0.1% Tween 20, then 5x PBS. All wash buffers are kept on ice.

3) DNA elution. DNA is recovered from the tubes with 2ml (equals coating volume) 50µg/ml Proteinase K/ 0.5% SDS in T.E. buffer for 30 minutes at 37°C. Eluted DNA is phenol-CCl3 extracted once then precipitated with 2µl 20mg/ml glycogen, 0.1 volumes 3M Sodium acetate, and 2-3 volumes of ice cold absolute ethanol, and pelleted at 13,000g (approx) for 20 minutes, and the pellet washed with 500µl 70% ethanol, then vacuum dried and re-suspended in 20µl DEPC treated water.

4) Full length PCR. All recovered DNA is used in 4 PCR reactions of 35 cycles. X174a* product is generated with 10pmol of each of TAC3 (1350bp product) and NNKGSLR2REC in a 100µl reaction, with 20µl of recovered DNA, and 2.5u of Taq DNA polymerase (Invitrogen) 200µM dNTPs, and 1x manufacturers buffer. PCR is then carried out for 35 cycles of 94°C, 45 seconds; 60°C, 1 minute; 72°C, 2 minutes, followed by 10 minutes at 72°C.

14. It follows that in a similar way, I would expect that any other cis-acting protein can be substituted for X174 or for P2A to create the library for selection.

15. Affinity selection is a facile mechanism for the identification of individual ligand encoding nucleic acids from very large, highly diverse gene libraries.

16. The physical linkage of each gene in a vast, diverse gene library with the polypeptide that it encodes is

central to the display technology concept of the present invention. The major benefit of this linkage is that it makes the isolation of individual sequences that encode polypeptides that bind to a given target compound facile.

17. The selection begins with the immobilisation of a target compound. Typically, this is done by adhesion of the target onto a plastic surface (e.g., a microtitre plate or plastic tube). Alternatively, the target could be biotinylated so that it, and anything that binds to it, can be recovered and separated from the non-binding material through capture onto streptavidin coated beads.

18. The library of polypeptide:nucleic acid complexes is then prepared: in the case of covalent display this would comprise *in vitro* generated covalent polypeptide:DNA complexes. This library is incubated with the target to allow the binding of those members of the library that recognise the target. Because the target is immobilised, bound members of the library become also immobilised. This allows non-binding members of the library to be removed by sequentially washing the selection tube with a pH buffered detergent/saline solution. Once washing is complete the bound library members are eluted from the target by adding an elution buffer to the selection tube. Typically this involves the addition of a solution containing a protease to release the encoding DNA molecule from the encoded, covalently attached, target-bound ligand. The released DNA is then re-amplified using PCR in order for the selection cycle to be repeated (thereby sequentially enriching specific peptides from residual non-specifically bound

sequences) and, ultimately, for the required DNA sequence to be isolated.

19. This selection mechanism does not therefore impose a limit on the number of genes sequences that can be addressed since individual clones are not screened, but rather, the whole library is interrogated *en masse* in a single selection vessel.

20. In summary, I can confirm that the substitution of alternative covalent cis-acting DNA binding proteins for P2A is a matter of routine and that the description provides sufficient information for selection and screening of the in vitro library so produced.

21. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States code, and that such willful false statement may jeopardise the validity of this declaration, the Patent application, or any Patents issuing thereon.

Kevin FitzGerald

This 24th Day of July 2003.

Curriculum Vitae

Kevin J FitzGerald

The Cobb
High Street, Hempstead
Essex
CB10 2PE, UK

Date of birth: 6th March 1964
Nationality: British
Marital status: Married

+44 (0) 1799 599001 (Home)
+44 (0) 7801 186355 (Mobile)
kevin.fitzgerald@isogenica.com

Employment History

April 2000 – present
Isogenica Ltd., Cambridge, UK

Chief Executive Officer

Founded Isogenica in April 2000. Formulated the strategy, wrote the business plan, produced all the financial projections, presented to potential investors and succeeded in securing £1.8m to initiate operations in December 2000.

Responsible, since the company's inception, for leading the strategic, operational, financial and commercial management of the company. In less than two years, the company has grown to 10 staff, the company's proprietary CDT and CIS-display technologies have been reduced to practice, two new display technologies have been invented with accompanying patent applications filed and 3 corporate alliances have been secured (with Invitrogen Inc., San Diego, USA; Smiths Industries, London, UK; and Affitech AS, Oslo, Norway).

Established an executive management team; identified the company's current premises and negotiated rent and refurbishment costs; recruited a team of six post-doctoral senior research scientists and a research manager.

Chaired the Board of Directors for the first 18 months of the company's existence.

February 1999 – March 2000
Actinova Ltd., Cambridge, UK

Research Manager/Member of the Executive Management Team.

Contributed to the strategic and general management of the company through membership of Actinova's Executive Management Team;
Had full budgetary responsibility for the library technologies division of Actinova's business;
Responsible for identifying and evaluating relevant in-licensing opportunities and

significant involvement with the company's out-licensing activities.
Managed the technical and commercial development of the company's library display technologies;
Recruited and managed a team of 5 research scientists;

January 1992 - February 1999
Cambridge Antibody Technology Ltd., Cambridge UK

Group Leader (final position)
Among the founding group of scientists;
Made a significant contribution to the development of a powerful suite of technologies for the isolation and characterization of human antibodies;
Attained comprehensive technical expertise in such areas as molecular biology, protein biochemistry, immunochemistry, tissue culture, cell-based assays and *in vivo* studies;

As Group Leader:
Managed a group of five scientists and provided the main interface between several internal functional groups;
Managed collaborative projects conducted with industrial partners and academic groups;

Member of the business development team.

September 1991 - December 1992
Baylor College of Medicine, Houston, USA
Postgraduate training in molecular genetics/developmental biology.

September 1989-September 1990
Kennedy Institute of Rheumatology, London, UK
Undergraduate research into the molecular genetics of rheumatoid arthritis.

Education

April 2000 – September 2002
Aston Business School, Birmingham, UK

MBA (Distinction) - specialism in finance
Dissertation title: "Estimating The Intrinsic Value Of An Emerging, Privately-Owned Biotechnology Company".

Awarded prestigious **Sainsbury Management Fellowship** from the **Royal Academy of Engineering** to cover the MBA course fees.

April 1995 - March 1997
MRC Laboratory of Molecular Biology, Cambridge, UK

Ph.D. in Molecular Biology and Protein Biochemistry.
Thesis title: "Engineering Bivalent and Bispecific Diabodies for Therapy".
Supervisor: Dr Greg Winter CBE FRS.

The first year of the project (from April 1994 – March 1995) was conducted at Cambridge Antibody Technology Ltd. As neither the MRC or Cambridge Antibody Technology are degree awarding bodies, the degree was awarded by the University of East London.

September 1987 - June 1991
University of East London, London, UK

BSc (Hons.) in Applied Biology. Degree awarded: **First Class**
Awarded the Upjohn Ltd. Academic Prize

Publications

Odegrip, R., Coomber, D., Eldridge, B., Hederer, R., Kuhlman P.A., Ullman, C., **FitzGerald, K***. and McGregor, D. (2003). CIS display: *In vitro* selection of peptides from libraries of protein-DNA complexes. Submitted
*Corresponding author

McGregor, D., **FitzGerald, K.** (2003). On display: new *in vitro* visualisation methods are helping to identify new drugs. *Modern Drug Discovery* (March) 23-24.

Isaksen, M., **FitzgGerald, K.** (2001). Purification and analysis of antibody fragments using proteins L, A and LA. In *Antibody Engineering - A Laboratory Manual.* (Dubel, S., Kontermann, R, eds.) Chapter 5.5. Springer-Verlag, Heidelberg, Germany.

FitzGerald, K. (2000). *In vitro* display technologies - new tools for drug discovery. *Drug Discovery Today* **5**, 253- 258.

Manzke, O., ****FitzGerald, K., Holliger, P., Klock, J., Span, M., Fleischmann, B., Hescheler, J., Qinghua, J., Johnson, K., Diehl, V., Hoogenboom, H. & Bohlen, H. (1999). CD3 X anti-nitrophenyl bispecific diabodies: Universal immunotherapeutic tools for retargeting T cells to tumours. *International Journal of Cancer* **82**,700-708.
**joint first authors.

FitzGerald, K., Holliger, P. & Winter, G. (1997). Improved tumour targeting by disulphide stabilised diabodies expressed in *Pichia pastoris*. *Protein Engineering* **10**, 1125-1129.

FitzGerald, K., Chiswell, D., Earnshaw, J., Smith, R., Kenten, J., Williams, R., McCafferty, J. (1996). Isolating high affinity human antibodies from phage repertoires. In *Combinatorial Libraries. Synthesis, Screening and Application Potential* (Cortese, R. ed.) pp. 189-204. Walter de Gruyter, Berlin, Germany.

McGuinness, B., Walter, G., **FitzGerald, K.**, Schuler, P., Mahoney, W., Duncan, A. & Hoogenboom, H. (1996). Phage diabody repertoires for selection of large numbers of bispecific antibody fragments. *Nature Biotechnology* **14**, 1149-1154.

Brown, C., **FitzGerald, K.**, Moyes, S., Mageed, R., Williams, D. & Maini, R. (1995). Immunoglobulin heavy chain variable region genes from the synovium of a rheumatoid arthritis patient shows little evidence of mutation but diverse CDR3. *Immunology* **84**, 367-374.

McCafferty, J., **FitzGerald, K.**, Earnshaw, J., Chiswell, D., Link, J., Smith, R. & Kenten, J. (1994). Selection and rapid purification of murine antibody fragments that bind a transition-state analogue by phage display. *Applied Biochemistry and Biotechnology* **47**, 157-173.

Patents

McGregor, D., Odegrip, R., Eldridge W. and **FitzGerald, K**. (2002). Display library (CIS display). UK patent application no. GB0220759.5

McGregor, D. and **FitzGerald, K**. (2002). Peptide library display method (liposome display). UK patent application no GB 0205008.6

Smith, R., McCafferty, J., Chiswell, D., Darsley, M., **FitzGerald, K.**, Kenten, J., Martin, M., Titmas, R., Williams, R. (1995). The isolation and production of catalytic antibodies using phage technology. International patent application no. W09527045.

Conferences

Oral presentations given at business/investor conferences:

BioPartnering Europe, London, UK (2001)
BioIndustry Association CEO/Investors, London, UK (2001 & 2002)
Science in the City, London, UK (2002)
Sachs-Bloomberg, London, UK (2002)
Sachs-Bloomberg, Munich, Germany (2002)
Atlas Ventures Annual Conference, Cannes, France (2002)
ERBI, Cambridge, UK (2002 & 2003)
Bluesky Investor Conference, Cambridge, UK (2003)

Oral presentations given at scientific conferences:

IBC Antibody Engineering, San Diego, USA (1999)
Phage Club, Montpellier, France (1999)
IBC Functional Proteomics, Boston, USA (1998)
Superantibodies, New Delhi, India (1998)
IRBM, Gubbio, Italy (1994)

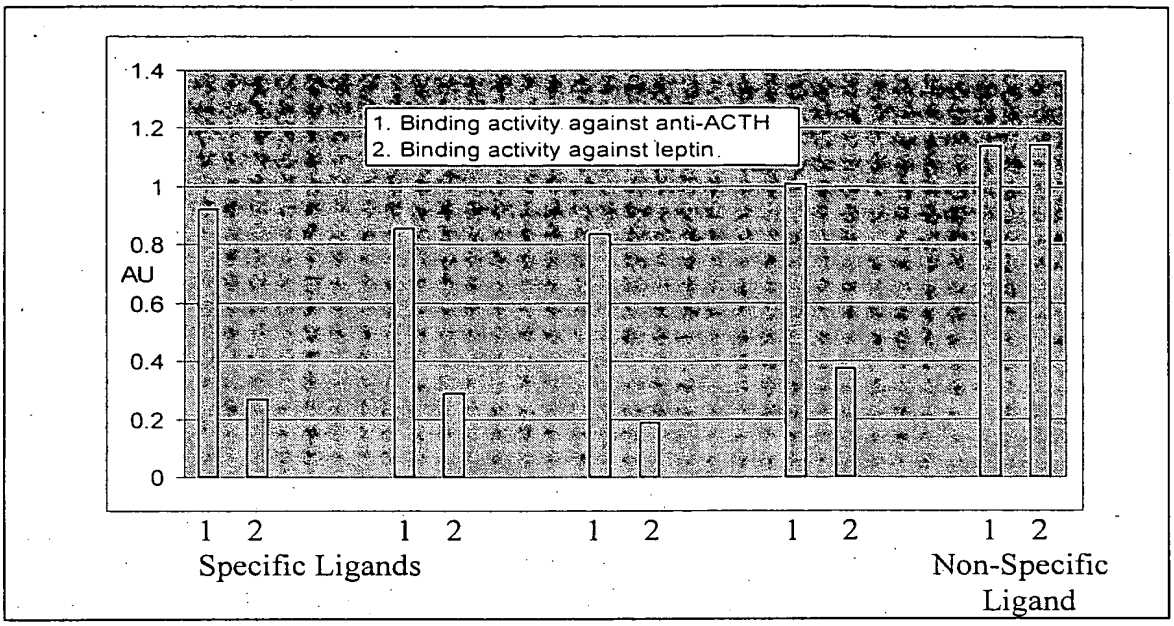

Figure 1: Identification of anti-ACTH peptide ligands from a ϕX174A CDT library.

DNA recovered following several rounds of CDT selection was cloned into an expression vector and peptides were produced by bacterial fermentation. The binding activities of the expressed peptides against anti-ACTH antibody (1) or the negative control leptin (2) were assessed by ELISA. Four clones were identified that bound specifically to anti-ACTH. One peptide was identified that bound to both anti-ACTH antibody and to leptin (i.e. a non-specific ligand).